【ABSTRACT】

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The present invention relates to a carbon nanotube (CNT)-biosensor comprising a bio-receptor chemically or physicochemically attached to a high density CNT pattern having carboxyl group exposed, in which the bio-receptor is capable of binding to a target biomaterial, and a method for preparing the same.

According to the present invention, it is possible to fabricate CNT-biosensor comprising various bio-receptors chemically or physicochemically bonded to a CNT pattern having exposed carboxyl groups or a CNT pattern having the exposed carboxyl groups modified by various chemical functional groups.

【REPRESENTATIVE DRAWING】

FIG. 1

【KEY WORDS】

carbon nanotube (CNT), bio-receptor, biosensor, carboxyl groups, modification

【SPECIFICATION】

【TITLE】

Method for Manufacturing a Biosensor Using the High Density Carbon Nanotube Pattern

【BRIEF DESCRIPTION OF THE DRAWINGS】

FIG. 1 shows the process for binding of complementary DNA to a CNT-DNA chip prepared by attaching DNA having amine groups to a CNT pattern having exposed carboxyl groups.

FIG. 2 shows the process for binding of complementary DNA to a CNT-DNA chip prepared by modifying a CNT pattern having exposed carboxyl groups with amine groups and attaching DNA having carboxyl group as the terminal group thereto.

FIG. 3 is an XPS spectrum for phosphorous of the surface of the CNT pattern having chemically bonded DNA.

FIG. 4(a) shows a image of the substrate comprising CNT having exposed carboxyl groups fixed at a high density, before binding of DNA, (b) shows the result of the fluorescence detection upon hybridization with complementary DNA, after binding DNA having amine group as the terminal group thereto to the above CNT pattern and (c) shows the result of the fluorescence detection upon hybridization with non-complementary DNA.

FIG. 5(a) shows the result of the fluorescence detection upon hybridization with complementary DNA, after binding DNA having carboxyl group as the terminal group thereto to the CNT pattern modified with amine groups, and (b) shows the result of the fluorescence detection upon hybridization with non-complementary DNA.

【DETAILED DESCRIPTION OF THE INVENTION】

【OBJECT OF THE INVENTION】

【FIELD OF THE INVENTION AND BACKGROUND ART】

The present invention relates to a carbon nanotube (CNT)-biosensor comprising a bio-receptor chemically or physicochemically bonded to a high density CNT pattern having carboxyl group exposed, in which the bio-receptor is capable of binding to a target biomaterial, and a method for preparing the same.

Carbon nanotube (CNT) is an allotrope of carbon, which abundantly exists on the earth. They are a tubular material where a carbon atom is connected with other carbons in the form of a hexagonal honeycomb structure. Their diameter is in the range of nanometer ($1/10^9$ meter). CNT is known to have excellent mechanical properties, electrical selectivity, field emission properties and highly efficient hydrogen storage properties and be new and almost defect-free of all the existing materials.

Because of their properties of excellent structural rigidity, chemical stability, ability to act as either a conductor or semiconductor and a large ratio of length compared to diameter, CNT exhibits great applicability as a basic material of flat panel displays, transistors, energy reservoirs, etc., and as various electron devices with nanosize.

In order to apply such properties more diversely, the single-wall CNT has been cut into fine pieces using strong acid. The CNT pieces have mainly the -COOH chemical functional groups at a part of the cut ends and side walls. The properties of the CNT have been modified by chemical binding of various chemicals using these chemical functional groups. Further, there have been reported that the functional group of CNT was substituted with –SH group by chemical manipulation and patterned on a gold surface using the technique of

micro contact printing (Nan, X. *et al.*, *J. Colloid Interface Sci.*, 245:311-8, 2002) and that CNT was immobilized on surface in the form of a multilayered film using the electrostatic method (Rouse, J.H. *et al.*, *Nano Lett.*, 3:59-62, 2003). However, the former has disadvantages of the low CNT surface density and the weak binding power, and the latter also has a fatal disadvantage in that the patterning method for selective immobilization on the surface cannot be applied. Therefore, there is an urgent demand for developing a new type surface immobilizing method.

At the present, it has been known that the functions of 10 thousand genes among about 100 thousand human genes come out into the open and most of the genes directly relate to diseases. Also, medical materials over 95% which are developed or are being developing target protein because most of the diseases are caused in protein level not DNA level. Therefore, on the basis of data obtained by protein function analysis, network analysis and function analysis of bio-molecule interacting with specific protein and ligand, the detection technique of the protein-protein reaction and protein-ligand reaction is necessary to study treating and preventing the diseases which have been impossible to treat and prevent by the conventional method.

Recently, researches are being conducted to detect reactions by means of electrochemical changes of CNT attached biomaterials, using electrical properties, semiconductor properties and structural stable properties of CNT(Dai, H. *et al.*, *ACC. Chem. Res.*, 35:1035-44, 2002; Sotiropoulou, S. *et al.*, *Anal. Bioanal. Chem.*, 375:103-5, 2003; Erlanger, B.F. *et al.*, *Nano Lett.*, 1:465-7, 2001; Azamian, B.R. *et al.*, *JACS*, 124:12664-5, 2002).

A representative example of a protein-ligand reaction is an avidin-biotin reaction. It is about to form a channel on a substrate, which had been treated with a polymer, using CNT and measure the binding activity of streptoavidin according to an electrochemical method (Star, A. *et al.*, *Nano Lett.*, 3:459-63, 2003).

The reasons that CNT attracts public attention as a biosensor are as

followings: Firstly, it needs no labeling; secondly, it has high sensitivity to signal change; and thirdly, it is capable of reacting in an aqueous solution without modification of a protein. Combination of a new nanomaterial and a biological system will create important applied technologies in respective fields of disease diagnosis (hereditary diseases), proteomics and nanobiotechnology.

In order to develop a rapider and cheaper biosensor, many researches have been conducted on technologies of DNA hybridization detection. Various labeling technologies for detecting DNA hybridization have been developed. At the present, the fluorescent materials are generally used for labeling. A single DNA strand is fixed, which is capable of detecting complementary DNA and the single DNA strand detects the complementary DNA in solution, and a signal converter change a DNA hybridization signal to a specific signal which can be analyzed.

An effective surface treatment capable of increasing hybridization efficiency and simultaneously, removing the background from non-specific binding is required to detect the DNA hybridization effectively using the DNA chip. Many researches have been conducted to prepare a surface-treated DNA chip platform (*Anal. Biochem.*, 266:23-30, 1999; *Nuc. Acid. Res.*, 29:107, 2001).

Also, various methods for detecting DNA hybridization were developed, which include the scanometric methods, the colorimetric methods, the methods using nanoparticle, the methods using electrochemistry, and etc.(*Science*, 289:1757-60, 2000; *Anal. Biochem.*, 295:1-8, 2001; *Analyst.*, 127:803-8, 2002; *Anal. Bioanal. Chem.*, 375:287-93, 2003).

Many applications with CNT in the bioengineering field have recently been appeared, such as glucose biosensors, detecting protein, detecting a certain DNA sequence and the like (*Anal. Bioanal. Chem.,* 375:103-5, 2003; *Proc. Natl. Acad. Sci. USA,* 100(9):4984-9, 2003; *Anal. Bioanal. Chem.,* 375:287-93, 2003). Screening bio-molecules from multilayer based on CNT can increase the amount of immobilized bio-substances, such as DNAs and detecting sensitivity to the bio-substances, since the CNT has wide surface area and high electrical

conductivity.

At the present time, the most universal method for detecting the reaction result in a biochip is to use conventional fluorescent materials and isotopes (Toriba, A. *et al.*, *Biomed. Chromatography:BMC.*, 17:126-32, 2003; Raj, S.U. *et al.*, *Anal. Chim. Acta*, 484:1-14, 2003; Peggy, A.T. *et al.*, *J. Microbio. Meth.*, 53:221-33, 2003). However, as novel methods to easily and precisely measure an electrical or electrochemical signal are attempted, there are increased demand for CNT as a new material.

The methods comprising preparing a high density CNT multiplayer, attaching DNA thereon and detecting complementarily bonded DNA are useful in genotyping, mutation detection, pathogen identification and the like. It has been reported that PNA (peptide nucleic acid: DNA mimic) is position-specifically fixed on a single walled CNT and the complementary binding to target DNA is detected (Williams, K.A. *et al.*, *Nature*, 420:761, 2001). Also, there have been an example, in which an oligonucleotide was fixed on a CNT array by an electrochemical method and DNA was detected by guanidine oxidation (Li, J. *et al.*, *Nano Lett.*, 3:597-602, 2003). However, these methods do not apply CNT in fabrication and development of biochips.

In recent, a high capacity biomolecule detection sensor using CNT was disclosed (WO 03/016901 A1). This patent relates to a multi-channel type biochip produced by arranging a plurality of CNTs on a substrate using a chemical linker and attaching various types of receptors. It has a disadvantage of relative weakness to environmental changes.

Therefore, the present inventors have found a method for producing a CNT-biosensor by laminating CNT on a desired position by chemical bonding to form a high density CNT pattern having exposed carboxyl groups and chemically binding a bio-receptor to the CNT pattern, and completed the present invention.

【TECHNICAL OBJECT OF THE INVENTION】

It is an object of the present invention to provide a CNT-biosensor comprising a bio-receptor attached to a desired position on a high density CNT pattern laminated by chemical bonding and a method for preparing the same.

It is another object of the present invention to provide a method for detecting various target biomaterials capable of binding to or reacting with a bio-receptor using the CNT-biosensor.

【CONSTITUTION OF THE INVENTION】

To achieve the above object, the present invention provides a carbon nanotube (CNT)-biosensor comprising a bio-receptor chemically or physicochemically attached to a CNT pattern fixing on the substrate and having carboxyl group exposed, in which the bio-receptors have a chemical functional group capable of binding to carboxyl group.

The present invention also provides a CNT-biosensor which is prepared by modifying the CNT pattern fixing on the substrate and having a carboxyl group exposed on its surface with a chemical compound having both a functional group capable of binding to the carboxyl group and a chemical functional group selected from the group consisting of amino group, aldehyde group, hydroxyl group, thiol group and halogen, followed by chemically or physicochemically binding bio-receptors to the chemical functional group selected from the group consisting of amino group, aldehyde group, hydroxyl group, thiol group and halogen, exposed by the modification, in which the bio-receptors have a functional group capable of binding to the chemical functional group.

In the present invention, the CNT pattern may be prepared by the steps of: (a) reacting a substrate having amino groups exposed on the surface with CNT having exposed carboxyl groups to form a CNT single layer on the substrate surface by amide bond formation between the amino group and the carboxyl group; (b) reacting the CNT single layer with a diamine type organic compound to form an organic amine layer on the CNT single layer and reacting the organic

amine with the CNT having exposed carboxyl groups to laminate a CNT layer thereon; and (c) repeating the step (b) n times to form CNT layers and organic amine layers alternately laminated for n times, thereby forming a high density CNT pattern having exposed carboxyl groups.

The present invention also provides a method for producing a CNT-biosensor comprising a bio-receptor bonded to a CNT pattern, which comprises the steps of: (a) reacting a substrate having amino groups exposed on the surface with CNT having exposed carboxyl groups to form a CNT single layer on the substrate surface by amide bond formation between the amino group and the carboxyl group; (b) reacting the CNT single layer with a diamine type organic compound to form an organic amine layer on the CNT single layer and reacting the organic amine with the CNT having exposed carboxyl groups to laminate a CNT layer thereon; (c) repeating the step (b) n times to form CNT layers and organic amine layers alternately laminated for n times, thereby forming a high density CNT pattern having exposed carboxyl groups; and (d) chemically or physicochemically binding bio-receptors to the high density CNT pattern having exposed carboxyl groups, in which the bio-receptors have a chemical functional group capable of binding to the carboxyl group.

The present invention also provides a method for producing a CNT-biosensor comprising a bio-receptor bonded to a CNT pattern, which comprises the steps of: (a) reacting a substrate having amino groups exposed on the surface with CNT having exposed carboxyl groups to form a CNT single layer on the substrate surface by amide bond formation between the amino group and the carboxyl group; (b) reacting the CNT single layer with a diamine type organic compound to form an organic amine layer on the CNT single layer and reacting the organic amine with the CNT having exposed carboxyl groups to laminate a CNT layer thereon; (c) repeating the step (b) n times to form CNT layers and organic amine layers alternately laminated for n times, thereby

forming a high density CNT pattern having exposed carboxyl groups; (d) modifying the high density CNT pattern having a carboxyl group exposed on its surface to produce with a chemical compound having both a functional group capable of binding to the carboxyl group and a chemical functional group selected from the group consisting of amino group, aldehyde group, hydroxyl group, thiol group and halogen; and (e) chemically or physicochemically binding bio-receptors to the chemical functional group selected from the group consisting of amino group, aldehyde group, hydroxyl group, thiol group and halogen, exposed by the modification, in which the bio-receptors have a functional group capable of binding to the chemical functional group.

According to the present invention, the substrate having exposed amino functional groups on its surface may be prepared by treating the substrate with amino alkyloxysilane, the amino groups having exposed on the substrate surface may be exposed in a pattern to bind CNT at a desired position.

Also, in the present invention, the substrate having the amino groups exposed in a pattern may be prepared by forming a photoresist or organic supra-molecule pattern on a substrate having the exposed amino groups. CNT may be laminated or fixed on such pattern in the vertical or horizontal direction. In the case of a nanopattern of an organic supra-molecule, CNT is preferably fixed in the vertical direction.

The substrate having the amino groups exposed in a pattern is prepared by forming a photoresist pattern on a substrate having exposed amino groups using photolithography which is conventionally used in the semiconductor process, or by forming a photoresist or organic supra-molecule pattern on a substrate, followed by treating with amino alkyloxysilane.

The present invention also provides a method for detecting a target biomaterial capable of binding to or reacting with a bio-receptor using the CNT-biosensor.

The present invention also a CNT-DNA chip, wherein the bio-receptor is DNA and a method for detecting DNA hybridization comprising using the CNT-DNA chip.

According to the present invention, the chemical functional group capable of binding to carboxyl group is preferably amino group or hydroxyl group.

According to the present invention, the bio-receptor may be, for example, enzyme substrates, ligands, amino acids, peptides, proteins, nucleic acid (DNA, RNA), lipids, cofactors or carbohydrate, which have carboxyl group, amino group, hydroxyl group, aldehyde group, or thiol group.

According to the present invention, the target biomaterial may be a substance able to serve as a target reacting with or binding to the bio-receptor to be detected, including preferably proteins, nucleic acids, antibodies, enzymes, hydrocarbons, lipids or other biomolecules derived from living bodies, more preferably nucleic acid (DNA, RNA) or proteins.

The term "CNT-biosensor" used herein inclusively refers to composites having a bio-receptor chemically or physicochemically bonded to a CNT pattern and may be defined as biochips comprising a bio-receptor attached to a high density CNT pattern by chemical or physicochemical bonding (particularly, amide bond).

According to the present invention, the substrate may be selected from the group consisting of silicone, glass, melted silica, plastics and PDMS (polydimethylsiloxane).

According to the present invention, the CNT-biosensor capable of detecting various types of target biomaterials directly or by an electrochemical signal is fabricated by repeatedly laminating CNT on a solid substrate coated with a chemical functional group (amino group) by chemical bonding to prepare a high surface density CNT pattern having exposed carboxyl groups and attaching a

bio-receptor having a functional group (amino group, hydroxyl group, etc.) capable of chemically reacting with the carboxyl group to the produced CNT pattern.

According to the present invention, by overcoming the limit of the conventional technologies in growing CNT using a catalyst fixed at a predetermined position, it is possible to form a pattern in a desired shape at a desired position. Also, the present invention improves the defects involved in the conventional technologies by forming a pattern on a substrate using a polymer or an organic supra-molecule so as to utilize chemical methods at maximum.

According to the present invention, the biosensor is fabricated by chemically or physicochemically binding a bio-receptor having a amino group or hydroxyl group capable of chemically reacting with the carboxyl group to the high density CNT pattern having exposed carboxyl groups.

Also, the biosensor is fabricated by chemically or physicochemically binding a bio-receptor having a carboxyl group or aldehyde group capable of chemically reacting with the amino group to the high density CNT pattern having exposed amino groups.

Meanwhile, in order to attach a bio-receptor without having a functional group capable of binding to the carboxyl group or amino group, the CNT pattern having the exposed carboxyl group is modified with a chemical having both a chemical functional group capable of binding to the carboxyl group and a chemical functional group capable of binding to the functional group of the target bio-receptor. Therefore, nearly all bio-receptors can be chemically or physicochemically attached to the high density CNT pattern.

For example, in order to attach a bio-receptor having thiol group, a CNT pattern is firstly modified with a chemical having both a chemical functional group capable of binding to the carboxyl group and the thiol functional group so that the thiol functional group is exposed on the surface of the CNT pattern.

Then, a bio-receptor having a thiol group is attached to the CNT pattern by S-S bond formation.

According to the present invention, the CNT may be connected through at least one conductive nanowire to an electric power source so that charge can be applied, in which the conductive nanowire may be formed as a single atom according to a conventional technology (*Science*, 275:1896-97, 1997), by forming a predetermined pattern of a conductive metal and depositing a wire, through which an electric current can flow, by ion implantation or sputtering.

The present invention will hereinafter be described in further detail by examples. However, it is to be understood that these examples can be modified into other various forms, and the scope of the present invention is not intended to be limited to such examples. Such examples are given to more fully describe the present invention for a person skilled in the art.

Example 1: Preparation of CNT having exposed carboxyl groups

The CNT which can be used in the present invention is not particularly limited and may be commercially available products or prepared by a conventional method. Pure CNT should be carboxylated at its surface and/or both ends to be used in the present invention.

The CNT having exposed carboxyl groups was refluxed in a sonicator containing a strong acid (a mixture of nitric acid and sulfuric acid for 24 hours and filtered through a 0.2μm filter. The residue was dipped in a condensed acid, heated to reflux at 90 for 45 hours and centrifuged. The supernatant was collected, filtered through a 0.1μm filter, and dried. The dried CNT having exposed carboxyl groups was dispersed in distilled water or an organic solvent, filtered through a 0.1μm filter to obtain CNT with a predetermined size.

Example 2: Preparation of a substrate having exposed amino group in a

pattern

In order to expose amino groups in a pattern on a substrate, 2 types of methods may be used. The first method includes forming a polymer, photoresist or organic supra-molecular pattern on a substrate such as silicone, glass, melted silica, plastics, PDMS (polydimethylsiloxane) and fixing amino alkyloxysilane on the substrate surface using the formed pattern as a mask to expose amino groups in a pattern on the substrate surface. The second method includes treating a substrate surface with amino alkyloxysilane and forming a polymeric photoresist or organic supra-molecular pattern to expose amino groups in a pattern on the substrate surface. A preferred example of amino alkyloxysilane is amino propyltriethoxysilane.

The above polymer or photoresist pattern may be easily fabricated by the method of photolithography which is conventionally used in semiconductor process. The organic supra-molecular pattern may be fabricated by the method of KR10-2003-0032514 and KR10-2003-0037752 which the present inventors already applied for.

Example 3: Preparation of a high density CNT pattern

The CNT having exposed carboxyl groups, prepared in Example 1, was reacted with the substrate having exposed amino groups in a pattern, prepared in Example 2 to form a CNT single layer on the substrate by amide bond formation between the carboxyl group and the amino group.

To accelerate the above amide bond, DCC(1,3-dicyclohexyl carbodiimide), HATU(*O*-(7-azabenzotriazol-1-yl)-1,1:3,3-tetra methyluronium hexafluorphosphate), HBTU(*O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate),
HAPyU(*O*-(7-azabenzotriazol-1-yl)-1,1:3,3-bis(tetramethylene)uronium hexafluorphosphate),
HAMDU(*O*-(7-azabenzotriazol-1-yl)-1,3-dimethyl-1,3-dimethyleneuronium hexafluorphosphate) or

HBMDU(*O*-(benzotriazol-1-yl)-1,3-dimethyl-1,3-dimethyleneuronium hexafluorphosphate) is preferably used as a coupling agent, and DIEA(diisopropylethylamine), TMP(2,4,6-trimethylpyridine), or NMI(*N*-methylimidazole) is preferably used as a co-coupling agent. Also, in the case of use water as solvent, EDC(1-ethyl-3-(3-dimethylamini-propyl) arbodiimide hydrochloride) is preferably used as a coupling agent, and NHS(*N*-hydroxysuccinimide) or NHSS(N-hydroxysulfosuccinimide) is preferably used as a co-coupling agent. The coupling agent participates in the formation of the amide bond (-CONH-) between the -COOH functional group and the $-NH_2$ functional group, and the base and the co-coupling agent act to increase the efficiency when the coupling agent forms the amide bond.

Then, the CNT attached to the substrate by amide bond was reacted with a diamine type organic compound having double amino functional groups while CNT having exposed carboxyl groups was reacted with amino groups at the other side of the diamine type organic compound.

The diamine type organic compound which can be used in the present invention includes compounds having a formula of HN_2-R_1-NH_2, in which R_1 is C_{1-20} saturated hydrocarbons un-saturated hydrocarbons or aromatic organic group.

Next, the chemical reaction between the CNT having exposed carboxyl groups and the diamine type organic compound was repeated to prepare a high density CNT pattern comprising the CNT layer and the organic amine layer alternately laminated for n times and having carboxyl groups exposed on its surface .

The CNT pattern having exposed carboxyl groups may be modified by chemicals having both a chemical functional group (amino group, hydroxyl group, etc.) capable of reacting with the carboxyl group and a chemical functional group (amino group, hydroxyl group, thiol group, aldehyde group, etc.) capable of binding to a functional group of a desired bio-receptor. The chemicals which can be used in such modification include HN_2-R_1-NH_2,

NN_2-R_2-SH, HN_2-R_3-OH, HN_2-R_4-CHO and the like, in which R_1, R_2, R_3 and R_4 are independently a C_{1-20} saturated hydrocarbon, un-saturated hydrocarbon or aromatic organic group.

Example 4: Fabrication of CNT-DNA chip

A DNA chip was fabricated by chemically attaching DNA to the CNT pattern having exposed carboxyl groups. A DNA chip was fabricated by attaching amino groups of DNA to the CNT pattern having exposed carboxyl groups, prepared in Example 2 (FIG. 1). Alternatively, a DNA chip may be fabricated by modifying the CNT pattern having exposed carboxyl groups, prepared in Example 2, with a diamine type organic compound having amino functional groups at both sides to expose amino functional groups and attaching carboxyl groups of DNA to the amino groups (FIG. 2). In this Example, a CNT-DNA chip was fabricated using oligonucleotide having the following SEQ ID NO: 1 having amino group or carboxyl group as the terminal group.

SEQ ID NO: 1 : 5'-TGT GCC ACC TAC AAG CTG TG(C3)-3'

The existing of DNA to the CNT pattern was confirmed by XPS (X-ray photoelectron spectroscope) spectrum for phosphorous atom considering the fact that all DNAs have phosphate groups (FIG. 3). As shown in FIG. 3, phosphorus was detected in the XPS surface analysis and thus, it was confirmed that DNA was attached to the CNT surface.

Example 5: Hybridization analysis using a CNT-DNA chip

The DNA chip prepared in Example 3 was placed in a hybridization chamber and a hybridization solution was drop with a pipette where the CNT had been fixed. Then, a cover slide was placed thereon. Here, the hybridization solution was mixed with 32 μℓ of a solution containing an oligonucleotide of complementary sequence to a total volume of 40 μℓ at a final concentration

3XSSC (0.45M NaCl, 0.045M sodium citrate) and 0.3% SDS(sodium dodecyl sulfate). The complementary oligonucleotide sequence was the following SEQ ID NO 2 having FITC (fluorescein isothiocyanate) attached to its end.

SEQ ID NO 2: 5'- CAC AGC TTG TAG GTG GCA CA-3'

The solution was left at 100 for 2 minutes and centrifuged for 2 minutes at 12000rpm to remove non-specific binding of two oligonucleotide strands. In order to prevent the hybridization solution from being dried in the hybridization chamber, 30 μℓ of 3XSSC (0.45M NaCl, 0.045M sodium citrate) was placed in hollows at both sides of the chamber and the chamber was closed and hybridized for 10 hours at 55 in a incubator.

After 10 hours, the hybridization chamber was took out of the incubator and immersed in 2XSSC solution for 2 minutes, then immersed in solution of 0.1XSSC (0.015M NaCl, 0.0015M sodium citrate) and 0.1% SDS for 5 minutes and finally was immersed in 0.1XSCC for 5 minutes. In order to remove remaining solution on DNA chip, the chip was placed in a centrifuge and centrifuged for 5 minutes at 600rpm.

The hybridization was detected through a fluorescent image using FITC labeled at the end of the oligonucleotide of the SEQ ID NO 2. The fluorescent image was obtained using ScanArray 5000 (Packard BioScience, BioChip Tecnologies LLC) confocal microscope and the QuantArray Microarray Analysis Software (FIG. 4). FIG. 4(a) shows a fluorescent image of the substrate comprising CNT having exposed carboxyl groups fixed thereon at a high density, before binding of DNA, (b) shows the result of the fluorescence detection upon hybridization with complementary DNA, after binding DNA having amine group as the terminal group thereto to the above CNT pattern (c) shows the result of the fluorescence detection upon hybridization with non-complementary DNA. It was confirmed that the fluorescence was clear and even when the oligonucleotide having the sequence complementary to the CNT pattern was hybridized (b). However, in the CNT pattern without having the oligonucleotide fixed thereon

and in the CNT-DNA chip hybridized with the oligonucleotide having the non-complementary sequence ((a) and (c)), no fluorescence was observed. From these results, it was confirmed that the non-specific reaction almost never occurred.

Example 6

As shown in FIG 2., a DNA chip was fabricated by chemically attaching DNA strand (sequence 1) having carboxyl group as the terminal group to the CNT which is modified with amino group. In FIG. 5, (a) shows the result of the fluorescence detection upon hybridization with complementary DNA and (b) shows the result of the fluorescence detection upon hybridization with non-complementary DNA. As a result of hybridization of the oligonucleotide having the non-complementary sequence with the oligonucleotide having the complementary sequence, it was possible to certainly distinguish between the hybridized sample and the non-hybridized sample.

【EFFECT OF THE INVENTION】

As described above, the present invention provides a biosensor comprising a bio-receptor chemically bonded to a high density CNT pattern produced by laminating CNT having exposed carboxyl groups at a desired position on a substrate.

According to the present invention, it is possible to fabricate CNT-biosensor comprising various bio-receptors chemically or physicochemically bonded to a CNT pattern having exposed carboxyl groups or a CNT pattern having the exposed carboxyl groups modified by various chemical functional groups.

【THE CLAIMS】

【CLAIM 1】

A carbon nanotube (CNT)-biosensor comprising bio-receptors chemically or physicochemically attached to a CNT pattern fixing with high density on the substrate and having carboxyl group exposed, in which the bio-receptors have a chemical functional group capable of binding to the carboxyl group.

【CLAIM 2】

A CNT-biosensor which is prepared by modifying the CNT pattern fixing with high density on the substrate and having a carboxyl group exposed on its surface with a chemical compound having both a functional group capable of binding to the carboxyl group and a chemical functional group selected from the group consisting of amino group, aldehyde group, hydroxyl group, thiol group and halogen, followed by chemically or physicochemically binding bio-receptors to the chemical functional group selected from the group consisting of amino group, aldehyde group, hydroxyl group, thiol group and halogen, exposed by the modification, in which the bio-receptors have a functional group capable of binding to the chemical functional group.

【CLAIM 3】

The CNT-biosensor according to claim 1 or 2, wherein the bio-receptor is an enzyme substrate, a ligand, an amino acid, a peptide, a protein, nucleic acid (DNA, RNA), lipid, a cofactor or carbohydrate.

【CLAIM 4】

The CNT-biosensor according to claim 3, wherein the bio-receptor is a protein or nucleic acid (DNA, RNA).

【CLAIM 5】

The CNT-biosensor according to claim 1 or 2, wherein the chemical functional group capable of binding to carboxyl group is amino group or hydroxyl group.

【CLAIM 6】

The CNT-biosensor according to claim 1 or 2, wherein the CNT-pattern is prepared by the steps of:

(a) reacting a substrate having amino groups exposed on the surface with CNT having exposed carboxyl groups to form a CNT single layer on the substrate surface by amide bond formation between the amino group and the carboxyl group;

(b) reacting the CNT single layer with a diamine type organic compound to form an organic amine layer on the CNT single layer and reacting the organic amine with the CNT having exposed carboxyl groups to laminate a CNT layer thereon; and

(c) repeating the step (b) n times to form CNT layers and organic amine layers alternately laminated for n times, thereby forming a high density CNT pattern having exposed carboxyl groups.

【CLAIM 7】

The CNT-biosensor according to claim 6, wherein the substrate having exposed amino functional groups on its surface is prepared by treating the substrate with amino alkyloxysilane.

【CLAIM 8】

The CNT-biosensor according to claim 6, wherein the amino groups having exposed on the substrate surface is exposed in a pattern to bind CNT at a desired position.

【CLAIM 9】

A method for producing comprising bio-receptor bonded to a CNT pattern, which comprises the steps of:

(a) reacting a substrate having amino groups exposed on the surface with CNT having exposed carboxyl groups to form a CNT single layer on the substrate surface by amide bond formation between the amino group and the carboxyl group;

(b) reacting the CNT single layer with a diamine type organic compound to form an organic amine layer on the CNT single layer and reacting the organic amine with the CNT having exposed carboxyl groups to laminate a CNT layer thereon;

(c) repeating the step (b) n times to form CNT layers and organic amine layers alternately laminated for n times, thereby forming a high density CNT pattern having exposed carboxyl groups; and

(d) chemically or physicochemically binding bio-receptors to the high density CNT pattern having exposed carboxyl groups, in which the bio-receptors have a chemical functional group capable of binding to the carboxyl group.

【CLAIM 10】

A method for producing a CNT-biosensor comprising a bio-receptor bonded

to a CNT pattern, which comprises the steps of:

(a) reacting a substrate having amino groups exposed on the surface with CNT having exposed carboxyl groups to form a CNT single layer on the substrate surface by amide bond formation between the amino group and the carboxyl group;

(b) reacting the CNT single layer with a diamine type organic compound to form an organic amine layer on the CNT single layer and reacting the organic amine with the CNT having exposed carboxyl groups to laminate a CNT layer thereon;

(c) repeating the step (b) n times to form CNT layers and organic amine layers alternately laminated for n times, thereby forming a high density CNT pattern having exposed carboxyl groups;

(d) modifying the high density CNT pattern having a carboxyl group exposed on its surface with a chemical compound having both a functional group capable of binding to the carboxyl group and a chemical functional group selected from the group consisting of amino group, aldehyde group, hydroxyl group, thiol group and halogen; and

(e) chemically or physicochemically binding bio-receptors to the chemical functional group selected from the group consisting of amino group, aldehyde group, hydroxyl group, thiol group and halogen, exposed by the modification, in which the bio-receptors have a functional group capable of binding to the chemical functional group.

【CLAIM 11】

The method according to claim 9 or 10, wherein the substrate having exposed amino functional groups on its surface is prepared by treating the substrate with amino alkyloxysilane.

【CLAIM 12】

The method according to claim 9 or 10, wherein the amino groups having exposed on the substrate surface is exposed in a pattern to bind CNT at a desired position.

【CLAIM 13】

The method according to claim 12, wherein the pattern of amino groups is prepared by forming a photoresist or organic supra-molecule pattern on a substrate having the exposed amino groups.

【CLAIM 14】

The method according to claim 12, wherein the pattern of amino groups is prepared by forming a photoresist or organic supra-molecule pattern on a substrate, followed by treating with amino alkyloxysilane.

【CLAIM 15】

The method according to claim 9 or 10, wherein the chemical functional group capable of binding to carboxyl group is amino group or hydroxyl group.

【CLAIM 16】

The method according to claim 9 or 10, wherein the bio-receptor is an enzyme substrate, a ligand, an amino acid, a peptide, a protein, nucleic acid (DNA, RNA), lipid, a cofactor or carbohydrate.

【CLAIM 17】

A method for detecting a target biomaterial capable of binding to or reacting

with a bio-receptor using the CNT-biosensor of claim 1 or 2.

【CLAIM 18】

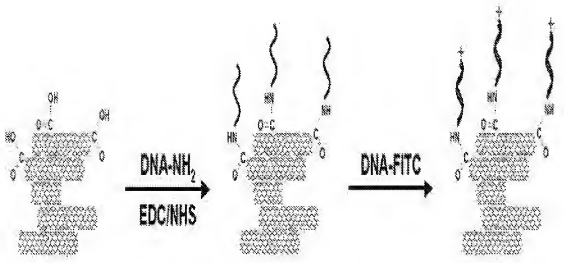
The CNT-DNA chip according to claim 1 or 2, wherein the bio-receptor is DNA.

【CLAIM 19】

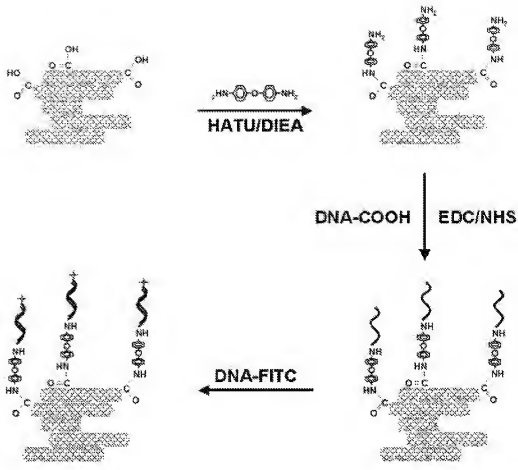
A method for detecting DNA hybridization comprising using the CNT-DNA chip of claim 18.

【DRAWINGS】

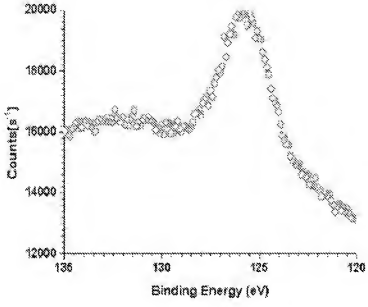
【FIG. 1】



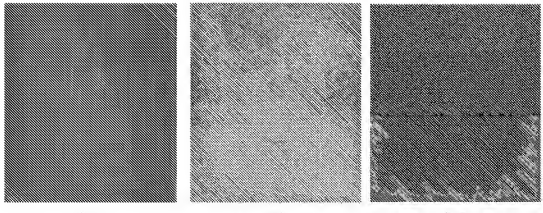
【FIG. 2】


HATU/DIEA
DNA-COOH
EDC/NHS
DNA-FITC

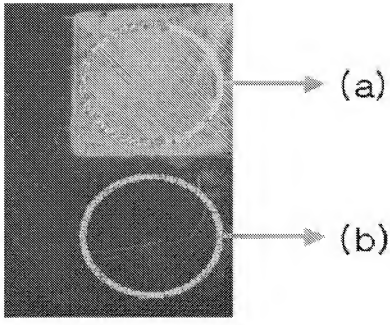
【FIG. 3】


20000
18000
16000
14000
12000
Counts[s⁻¹]
135
130
125
120
Binding Energy (eV)

【FIG. 4】


(a)
(b)
(c)

【FIG. 5】



【SEQUENCE LIST】

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<120> Method for Manufacturing a Biosensor Using the High Density Carbon Nanotube Pattern

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